THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 62:80–85, 1974

Vol. 62, No. 2
Printed in U.S.A.

THE EFFECTS OF FRICTIONAL STIMULATION ON MOUSE EAR EPIDERMIS. I. CELL PROLIFERATION*

IAN C. MACKENZIE, PH.D., B.D.S., F.D.S.R.C.S.†

ABSTRACT

The mitotic response of mouse ear epidermis to a controlled frictional stimulus produced by a rotating brush has been studied. A single application of friction resulted in an increased rate of cell proliferation judged by the number of Colcemid-arrested mitotic nuclei per unit length of epidermis. Maximum mitotic activity was found 48 hr after friction. Mitotic duration was apparently reduced in friction specimens. Daily application of friction for up to 35 days resulted in a high level of mitotic activity throughout the experimental period. The epidermal mitotic response to friction appears to be similar to the response to other forms of physical or chemical trauma.

A variety of mechanical stimuli, usually grouped together under the term friction, results in an adaptive response of the skin which leads to thickening and the formation of epidermal callus. This response is of general interest to those concerned with physiologic adaptation, and is of particular interest to the dermatologist, as the alteration which occurs in epidermal structure implies a change in the balance of homeostatic mechanisms which maintain the normal epidermal thickness. The response of the epidermis to repeated mechanical stimulation has not been comprehensively studied.

Naylor [1] has pointed out that, depending on the type and frequency of application, the epidermis responds to friction either by blistering, an acute response to friction, or by thickening, a chronic frictional response. There are several reports of studies of epidermal friction blisters [1–3] but experimental studies of the effects of chronic friction have been limited to a simple examination of the histologic changes which occur. Ruben [4] found that human skin, after mechanical stimulation for 30 days, showed thickening of the stratum corneum. He was unable to detect any increase in the thickness of the nucleated cell strata of the epidermis and he attributed thickening of the stratum corneum to a decreased rate of shedding of cells rather than an increased rate of cell proliferation. Rothman [5, 6] extended Ruben's hypothesis by suggesting that friction induces an alteration in the differentiation of the epidermis leading to the formation of a harder keratin like that of hair or nail which does not desquamate. Mercer [7] has postulated a mechanism by which such a change could be produced; a change in the differentiation of the epidermis following friction has not, however, been directly demonstrated.

Irrespective of changes which may occur in epithelial differentiation, there are indications that the epidermal response to friction may be associated with an increased rate of cell proliferation. Carter [8], examining the keratinizing epithelium of the rodent palate, found that friction produced an increase in the thickness of the stratum Malpighii of the epithelium, a change which implies either an increased rate of cell proliferation or a decreased rate of cell maturation. Mechanical stimuli, such as massage [9] or removal of the stratum corneum with adhesive tape [10–13] have been shown to increase the rate of cell proliferation.

To gain further information about the nature of the epithelial response to chronic mechanical stimulation, a series of experiments was undertaken to analyze the changes which occur in the rate of cell proliferation, histologic appearance, and number of layers and size of cells in the malpighian region and stratum corneum of mouse ear epidermis. The present paper describes the effects of chronic frictional stimulation on the rate of epithelial cell proliferation. The changes found in the histologic appearance of the epithelium and in the structure of the stratum corneum will be reported subsequently.

MATERIALS AND METHODS

Adult male Balb/C mice (body weight 24–28 gm) were used in all experiments, and friction was applied to the dorsal epidermis of the left ear within an area of approximately 15 mm^2 which was marked by tattooing with india ink 2 to 6 weeks before the application of friction. A similar area of the right ear served as control. Mice were immobilized without aid of a general anesthetic during the application of friction. The frictional stimulus applied was that produced by a rotating goat-hair brush (Rotifix, No. 84) driven by a variable-speed electric motor. The spindle of the brush lay within a vertical slot in a metal support and was interrupted by a length of coiled spring. During rotation, vertical deflection of the spring allowed the brush to move within the

Manuscript received July 20, 1973; in revised form September 20, 1973; accepted for publication October 10, 1973.

This work was supported by a grant from the Medical Research Council.

* From the Department of Oral Pathology, London Hospital Medical College, London, England. † Present address: Department of Oral Biology, College of Dentistry, University of Iowa, Iowa City, Iowa 52242.

TABLE I

Results of counts of Group B mice

	Mitosis A	Colcemid MF	Mitosis B	Mean Mit.	MR	MD_f/MC_c
Group 1 (9–15 hr after friction)						
F	3.0 ± 0.7	24.5 ± 5.9	7.3 ± 1.3	5.2	3.22	0.49
C	0.5 ± 0.6	11.0 ± 2.3	7.2 ± 1.1	3.9	1.18	
Group 2 (14–18 hr after friction)						
F	8.5 ± 1.5	18.5 ± 1.2	7.3 ± 1.4	7.9	2.65	0.14
C	7.3 ± 1.1	7.2 ± 1.1	4.8 ± 1.0	6.1	0.28	

Results show (1) the number of metaphase figures arrested by Colcemid (Colcemid MF) during the periods 9–15 and 14–18 hr after friction, (2) the mitotic index of mice at the beginning (Mitosis A) and end (Mitosis B) of each blocking period, (3) estimated mean mitotic index during the blocking period (Mean Mit.), (4) the calculated mitotic rate (MR), and (5) the estimated decrease in mitotic duration during these periods following friction (MD_f/MD_c).

range permitted by the slot and produced a steady force acting at the brush head.‡

A suitable frictional stimulus which produced epithelial thickening without histologically detectable epidermal damage was determined in preliminary experiments. The stimulus used in all the experiments described here was 10 revolutions of the brush rotating at a speed of 40 or 50 rpm with a force of 8–9 gm.

Daily friction was applied so that for each particular experiment all mice received the final application of friction at the same time of the same day. At appropriate time intervals thereafter, groups of mice were killed and the ears removed. Each friction specimen was paired with a control specimen from the same animal so that calculations of the alteration of mitotic rate produced by friction were free of errors introduced by individual or diurnal variation. Differences between the control and friction specimen of each pair were analyzed using a pooled t-test. A paired t-test was employed to examine the significance of differences between groups of animals.

To arrest dividing cells in metaphase, mice were injected intraperitoneally 6 hr before death with Colcemid (Ciba) at a dose rate of 0.2 mg/100 gm body weight. Control and friction specimens, for histologic examination and cell counts, were fixed in Bouin's solution, processed for wax embedding, serially sectioned at 6 μm, and stained with celestine blue and eosin.

Mouse ear epidermis has a flat dermoepidermal junction, and counts of metaphase figures were made per unit length of epidermis. The unit of measurement employed was the diameter of a 100× oil immersion lens (0.16 mm). For each specimen, 10 consecutive fields of vision of each of 12 sections (Groups A and C below) or 10 sections (Group B below) were counted. At least two sections separated each of the sections counted. Three groups of mice were investigated:

Group A. To examine the effect of a single application of friction on the rate of epidermal cell proliferation, 48 mice received a single application of friction and groups of 3 mice were killed at 6-hr intervals, 6 to 96 hr later.

Group B. To examine the effect of friction on epidermal mitotic duration, 24 mice received applications of friction on three consecutive days. At 9 and 14 hr following the final application of friction, 4 mice were killed and 4 injected with Colcemid. Subsequently, the Colcemid-injected mice and a further 4 uninjected mice were killed. The schedule of treatment of these animals and the time of death with respect to the application of friction and injection of Colcemid is shown in Table I.

Group C. To examine the effects of repeated daily application of friction to the epidermis, 40 mice received daily friction for 1, 7, 14, 28, or 35 days and were killed 6, 12, 18, or 24 hr after the final application of friction.

RESULTS

Group A. The results of counts of metaphase figures per unit length of epidermis 6–96 hr after a single application of friction are shown in Figure 1. Six and 18 hr after the application of friction, fewer metaphase figures were found in friction specimens than controls, but at all subsequent times the mean number of metaphase figures was higher in friction specimens. Maximum mitotic activity in friction specimens occurred during the period 30–60 hr after the application of friction and peak activity was found in the 48-hr specimens. The mean number of metaphase figures for the entire 96-hr period was 2.2 times greater for friction specimens (friction = 3.82, control = 1.76, by paired t-test, $p < 0.01$).

Group B. The results of counts of mitotic nuclei per unit length of control and friction epidermis of Group B mice are shown in Table I. For all time periods, the rate of accumulation of metaphase figures in Colcemid-injected mice and the number of mitotic nuclei in uninjected mice were greater in the friction specimens. A marked difference was found between the number of mitotic nuclei at the beginning and at the end of each blocking period in specimens of epidermis from groups of uninjected mice; this difference was greater in unrubbed epidermis. This finding of an alteration in mitotic index in uninjected animals during the time periods used for Colcemid-blocking invalidates assumption of a steady rate of accumulation of metaphase figures under the action of Colcemid; for this reason the results were unsuitable for estimating real values for mitotic duration. An indication of

‡ Mackenzie IC: An experimental study of the effects of mechanical stimulation on keratinizing epithelia of rodents. Ph.D. Thesis, University of London, 1970

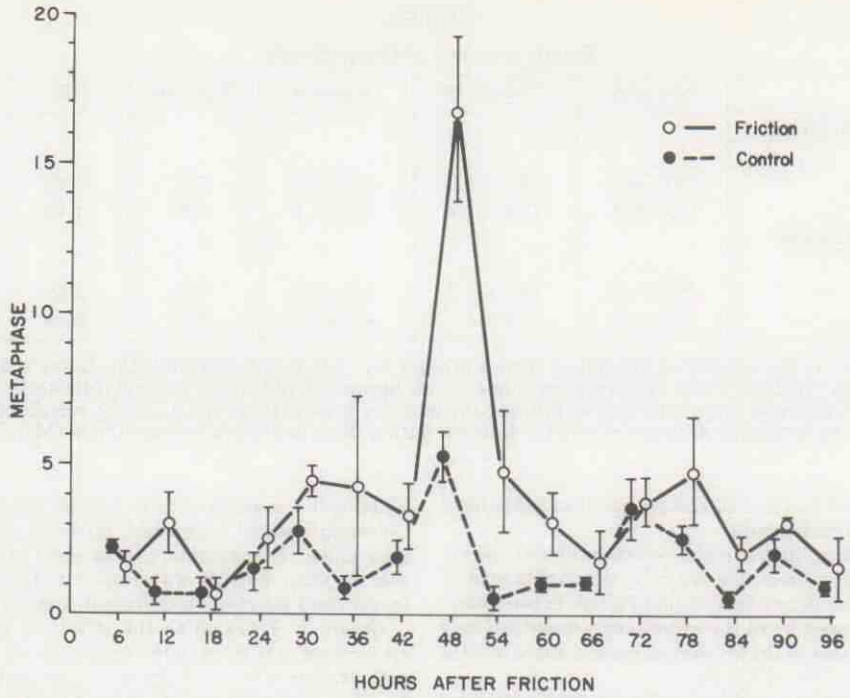

FIG. 1: Number of metaphase figures per unit length of mouse ear epidermis 6–96 hr after a single application of friction (Group A mice). Each point represents the mean of 3 animals ± one standard error. In all figures, the time indicated refers to the time at which specimens were taken and represents the number of metaphase figures arrested by Colcemid during the preceeding 6 hr.

the relative duration of mitosis in control and friction specimens (MD_c, MD_f) was obtained by substituting the observed values for rate of accumulation of metaphase figures under the action of Colcemid (MR_c, MR_f) and mean mitotic index during the blocking period (MI_c, MI_f) in the formula, derived from Iversen [15]:

$$MD_f/MD_c = MI_f\ MR_c/MI_c\ MR_f$$

The values obtained in this way indicated a reduction in mitotic duration in the epidermis of friction specimens during the periods 9–15 hr and 14–18 hr after the application of friction (Table I).

Group C. The results of counts of metaphase figures in the epidermis of mice killed at 6-hr intervals during the 24-hr period following 1–35 days of friction are shown in Table II. Mitotic activity was initially reduced in friction specimens during the 24 hr following a single application of friction, but comparison of the friction and control specimens from mice killed after 7, 14, 28, or 35 applications of daily friction showed a consistently greater number of metaphase figures in the rubbed epidermis; in 19 of the 28 pairs of specimens this difference was statistically significant (pooled t-test for each animal, $p < 0.01$). By grouping the results for mice killed during the 24-hr period after the final application of friction, the daily mitotic activity of the epidermis which had received 7, 14, 28, and 35 applications of friction was estimated to be 3.48, 5.63, 3.75, and 2.84 times higher than that of the corresponding control specimens (Table II). Grouping results for all mice by time after the final application of friction showed that mitotic activity in friction specimens was lowest 6–12 hr, and highest 12–18 hr, after the application of friction (Fig. 2).

Counts of metaphase figures in friction specimens from mice killed 24–48 hr after 1 or 35 applications of daily friction were greater than the corresponding counts for the period 0–24 hr after friction (Figs. 3, 4). During this time period, the mean number of metaphase figures in the 1- and 35-day friction specimens was 5.46 and 4.06 greater than in the corresponding controls (Table II).

DISCUSSION

The epidermal mitotic response to a single application of friction appears in several respects to be similar to the epidermal response to other forms of mechanical trauma. During the 24 hr following a single application of friction there was evidence of some depression of mitotic activity (Table II), maximum mitotic activity was found after 48 hr, and there was a return to approximately normal levels after four days. A similar initial depression of mitotic activity has been reported to occur after wounding human epidermis [16] and the epidermis of experimental animals [17]; epidermal mitotic activity has been reported to reach peak values during the period 24–60 hr after wounding [16, 17] or after removal of the

TABLE II

Number of metaphase figures per field of control and friction specimens of mouse ear epidermis following 1–35 applications of daily friction

Number of days of friction	1	1	7	14	28	35	35
Time period after friction (hours)	0–24	24–48	0–24	0–24	0–24	0–24	24–48
Number of animals	8	4	4	4	8	8	4
Mean metaphase per field (control)	2.36	2.40	0.85	1.48	0.88	1.83	2.31
Mean metaphase per field (friction)	1.24	13.11	2.96	8.33	3.30	5.19	9.38
Increase in metaphase (M_f/M_c)	0.53	5.46	3.48	5.63	3.75	2.84	4.06

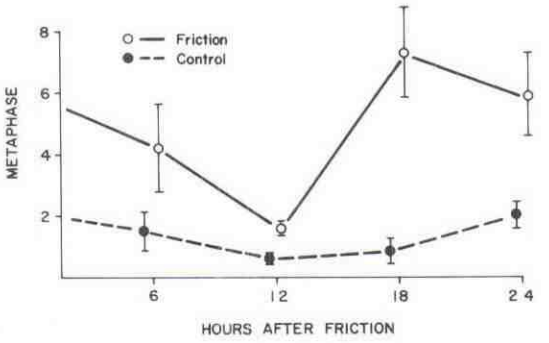

FIG. 2: Results of counts of metaphase figures per unit of epidermis which had received 1–35 applications of daily friction grouped by time after application of friction. Each point represents the mean of 8 mice ± standard error.

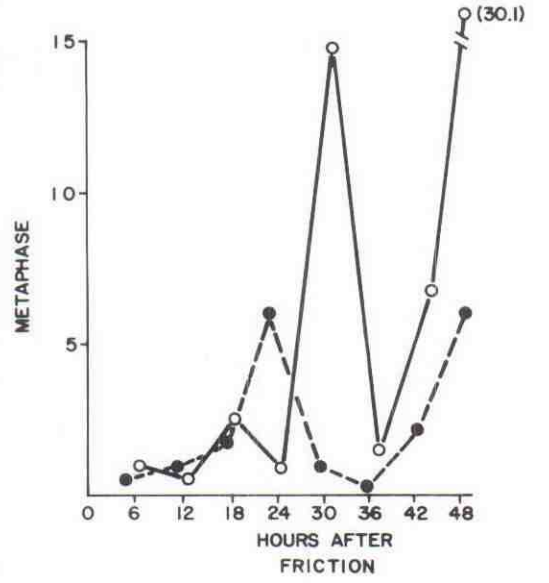

FIG. 3: Number of metaphase figures per unit length of mouse ear epidermis 6–48 hr after a single application of friction (Group C mice). The mitotic response with early depression of mitotic activity and high mitotic activity 48 hr after friction is similar to that shown in Fig. 1 (friction = continuous line).

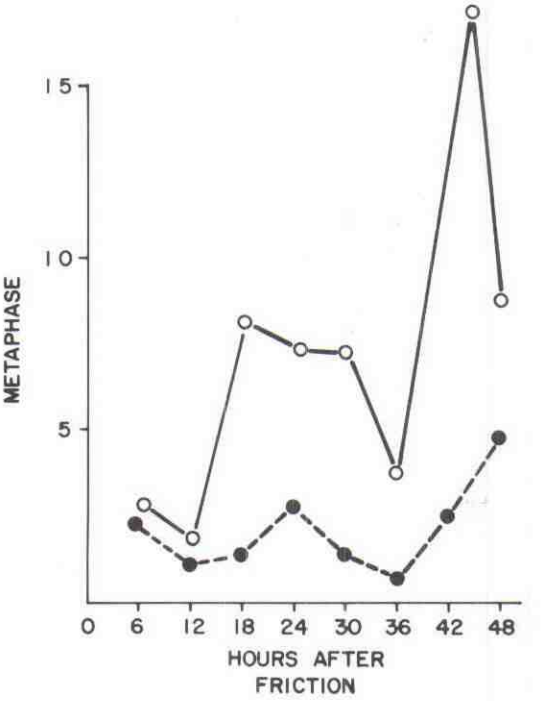

FIG. 4: Number of metaphase figures per unit length of mouse ear epidermis 6–48 hr following the 35th day of friction (Group C mice). The level of mitotic activity is similar to that shown in Figs. 1 and 2, but the response appears to be less delayed and an initial depression of mitosis below control levels is not seen (friction = continuous line).

stratum corneum with adhesive tape [10, 13]. An epidermal mitotic response showing initial depression, followed by a peak activity and gradual return to normal levels, is also seen after other, nonmechanical, forms of epidermal injury such as that produced by ultraviolet light [18, 19], carcinogens, and chemical irritants [20–22]. Goss [23] has described a similar pattern of mitotic activity to be a general feature of the response of internal organs such as the liver, thyroid, and kidney to injury or to an increased functional demand; this type of mitotic response may be typical of the majority of

tissues, including epidermis, which are capable of cell division.

The number of metaphase figures occurring per unit of length of epidermis depends not only on the mitotic rate of the tissue, but also upon the duration of mitosis. The effects of changes in mitotic duration on estimates of mitotic rate are greatly lessened by the use of cell-blocking agents such as Colcemid; nevertheless the effects of friction on mitotic duration were investigated to eliminate the possibility that the apparent increase in the mitotic rate of friction specimens resulted from a greatly extended mitotic duration. A change of this sort may be observed after the application of carcinogens [20]. Because of the variation of mitotic index in the unblocked mice during the time periods used to investigate mitotic duration, little confidence can be placed in the accuracy of the results obtained. However, the calculated degree of reduction appears sufficient to indicate that there was in fact a reduction, rather than an increase, in the epidermal mitotic rate as a result of the frictional stimulus. This would be in keeping with previous findings that an increased rate of cell proliferation is usually associated with a reduction in the duration of mitosis; epidermal mitotic duration varies inversely with diurnal variation in mitotic rate and with experimentally induced in vitro variations [24, 25] and is decreased during the periods of increased mitotic activity which follow wounding [17] and treatment with carcinogens [21].

In tissues receiving repeated friction, the mitotic rate remained high throughout the experimental period and there was no detectable trend towards either an increase or decrease in daily mitotic activity with increasing periods of application of friction. The mitotic response during the first week of daily application was not studied, but after seven days it appeared that each subsequent application of friction produced a similar mitotic response.

The cause of the increase in epidermal mitotic rate following friction is uncertain. Currently, the most informative approach to the problem of control of epidermal mitotic activity appears to be that of Bullough [14] who has explained homeostasis in terms of feedback of a mitosis-inhibiting chalone which is produced by postmitotic differentiating cells. In the present series of experiments, epidermal damage produced by the stimulus was not detected histologically, but more severe frictional stimuli have been shown to cause disruption of cells, particularly of the midspinous layer [1–3], and it has been shown that removal of the stratum corneum by stripping with adhesive tape results in cytologic changes throughout the stratum Malpighii of the epidermis [26]. It may be, therefore, that friction, by damaging cells of the deeper epidermal strata, results in either a depressed synthesis of chalone or its rapid loss due to increased membrane permeability which, by releasing the basal cells from mitotic inhibition, results in an increased rate of cell proliferation.

The present results indicate that the epidermal response to a frictional stimulus is associated with an increased rate of cell proliferation. A single application of friction produces a mitotic response which is essentially similar to that following other forms of mechanically or nonmechanically induced epidermal damage. With continuing daily friction, a high daily mitotic rate is maintained. These findings indicate that interpretation of the epidermal response to friction solely in terms of a change in tissue differentiation leading to a reduced rate of desquamation is unjustified.

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